Lysis of Fungal Hyphae in Soil and Its Possible Relation to Autolysis

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ABSTRACT

Living or dead hyphae of *Fusarium solani* f. *phaseoli*, *F. solani* f. *pisi*, *Glomerella cingulata*, *Helminthosporium victoriae*, *Mucor ramannianus*, *Penicillium frequentans*, and *Verticillium albo-atrum* were completely lysed on soil in 4-8 days, but some hyphae of *Rhizoctonia* spp. remained after 8 days. Fungi on sterilized soil grew well without lysis, but reinfesting the soil with certain streptomycetes or bacteria restored the lytic property. Amendment of soil with glucose or peptone temporarily annulled soil mycolysis, whereas chitin amendment enhanced mycolysis. Amendment of natural soil with living hyphae of *G. cingulata* resulted in a 70-fold increase in bacteria in 5 days and a 10-fold increase in actinomycetes in 9 days.

Living hyphae of *G. cingulata*, *H. victoriae*, and *F. solani* f. *phaseoli* lysed almost completely when separated from soil by a membrane filter which prevented passage of enzymes, but dead hyphae did not lyse at all. Hyphae of *G. cingulata* and *H. victoriae*, kept in a state of starvation on a dialysis tubing inflated with circulating water or mineral salt solution, autolyzed completely in 1-2 days after application of any of several antifungal antibiotics to the hyphae. Antibiotics or starvation conditions applied separately induced only a slight degree of autolysis. Antibiotic activity was extracted from soil previously supplemented with living mycelium of *G. cingulata*. In soil, fungal autolysis may be induced by a combination of starvation conditions and the presence of antibiotics in the vicinity of the fungus.

The property of soils to lyse fungi is apparently widespread (10, 11, 16, 17) and is relatively nonspecific although fungi differ in degree of susceptibility (11). Mycelia are most susceptible to lysis in soil, but spores may also be lysed. Soil mycolysis is associated with the presence of other microorganisms since this property is lacking in sterilized soil. Mycolysis may be restored by many actinomycetes (10, 12) and fewer bacteria (14, 16). Some of these microorganisms will lyse fungi in culture in the absence of soil (4, 9, 12, 14).

Much of the existing information on soil mycolysis was obtained by indirect methods such as spreading soil over agar cultures of fungi (10, 11, 12). A direct method of assay for soil fungistasis which utilizes a plastic peeling to remove fungal spores from a soil surface (7) was adapted for studying lysis of fungal hyphae on soil. Using this method, we have repeated and extended some earlier work using indirect methods. In addition, attempts were made to determine whether mycolysis in soil is autolytic or heterolytic.

We define mycolysis as loss of protoplasm and enzymatic dissolution of fungal cell walls. Hyphae containing segmented protoplasm and empty but intact hyphae formed by leakage or self-digestion of protoplasm are regarded as incompletely lysed, although these may represent stages in the complete lysis of living mycelium.

Materials and Methods.—Hyphae of test fungi (Table 1) were obtained by germinating conidia, sporangiospores, or chlamydospores for 4-16 hr in a medium containing 0.5% peptone and 0.5% glucose. Hyphae of *Rhizoctonia* spp. grown in the nutrient solution were prepared by gentle fragmentation in a Waring Blendor. Chlamydospores of *Fusarium solani* (Mart.) Appel & Wr. f. *phaseoli* (Burk.) Snyd. & Hans. were obtained from macroconidia after about 5 weeks in glass-distilled water. Hyphae of all fungi were washed three times in 1% Reynolds' salt solution (18) by low-speed centrifugation before use in lysis experiments. Fungus cultures were maintained in potato-dextrose agar.

The soil used, Conover loam, is an agricultural topsoil from a field left fallow for several years. No chemicals had been applied to the soil except for an annual broadcast application of 12-12-12 commercial fertilizer at the rate of 500 lb./acre. The soil was sieved and either used immediately after collecting it from the University farm or stored in glass jars as previously described (7). Nonsterilized Conover loam soil is referred to herein as natural soil.

Soil mycolysis was assayed by a direct method previously described in detail for assay of soil fungistasis (7). Natural soil, or sterilized soil reinfested with selected soil microorganisms, was adjusted to about 25% moisture content and compacted into small petri dishes, and the surface of the soil was smoothed with a spatula. Germinated spores or hyphal fragments of the test fungi were then applied uniformly over the soil surface. The plates were incubated with high humidity at 24 C for different time intervals, after which the hyphae were stained on the soil surface with rose bengal and recovered with plastic films made from a solution of polystyrene. Amount of soil mycolysis was estimated on a 0-4 scale: 0 = no lysis; 1 = 1-10% of hyphae lysed; 2 = 10-50% of hyphae lysed; 3 = 50-90% of hyphae lysed; and 4 = 90-100% of hyphae lysed (12). Hyphae recovered from soil immediately after deposition served as controls.

Several experiments required the use of sterilized

TABLE 1. Lysis of living and heat-killed hyphae of different fungi on natural soil and on sterilized soil infested with a streptomycete

	Lysis rating after 2, 4, and 8 days[a]								
	Living hyphae						Dead hyphae[c]		
	Natural soil			Streptomycete soil[b]			Natural soil		
Fungus	2	4	8	2	4	8	2	4	8
Glomerella cingulata	2	4	4	0	3	4	0	2	4
Mucor ramannianus	2	3	4	0	3	4	0	1	3
Helminthosporium victoriae	2	3	4	0	3	4	0	2	4
Penicillium frequentans	2	3	4	0	2	3	0	1	4
Fusarium solani f. *pisi*	0	3	4	0	2	3	0	1	4
F. solani f. *phaseoli*	0	3	4	0	2	3	0	1	4
F. solani f. *phaseoli*[d]	0	3	4						
Verticillium albo-atrum	0	2	4	0	2	3	0	1	4
Rhizoctonia solani	0	2	3	0	1	2	0	0	2
R. solani	0	1	3	0	1	2	0	0	2
R. praticola	0	1	3	0	1	2	0	0	3

[a] Lysis was rated 0-4: 0 = no lysis; 1 = 1-10% of mycelium lysed; 2 = 10-50%; 3 = 50-90%; 4 = 90-100%. Values are from one of three experiments with comparable results.
[b] Autoclaved soil infested with a streptomycete isolate.
[c] Fungal mycelia were killed by exposure to 80 C for 4 min.
[d] Germinated chlamydospores of *F. solani* f. *phaseoli*.

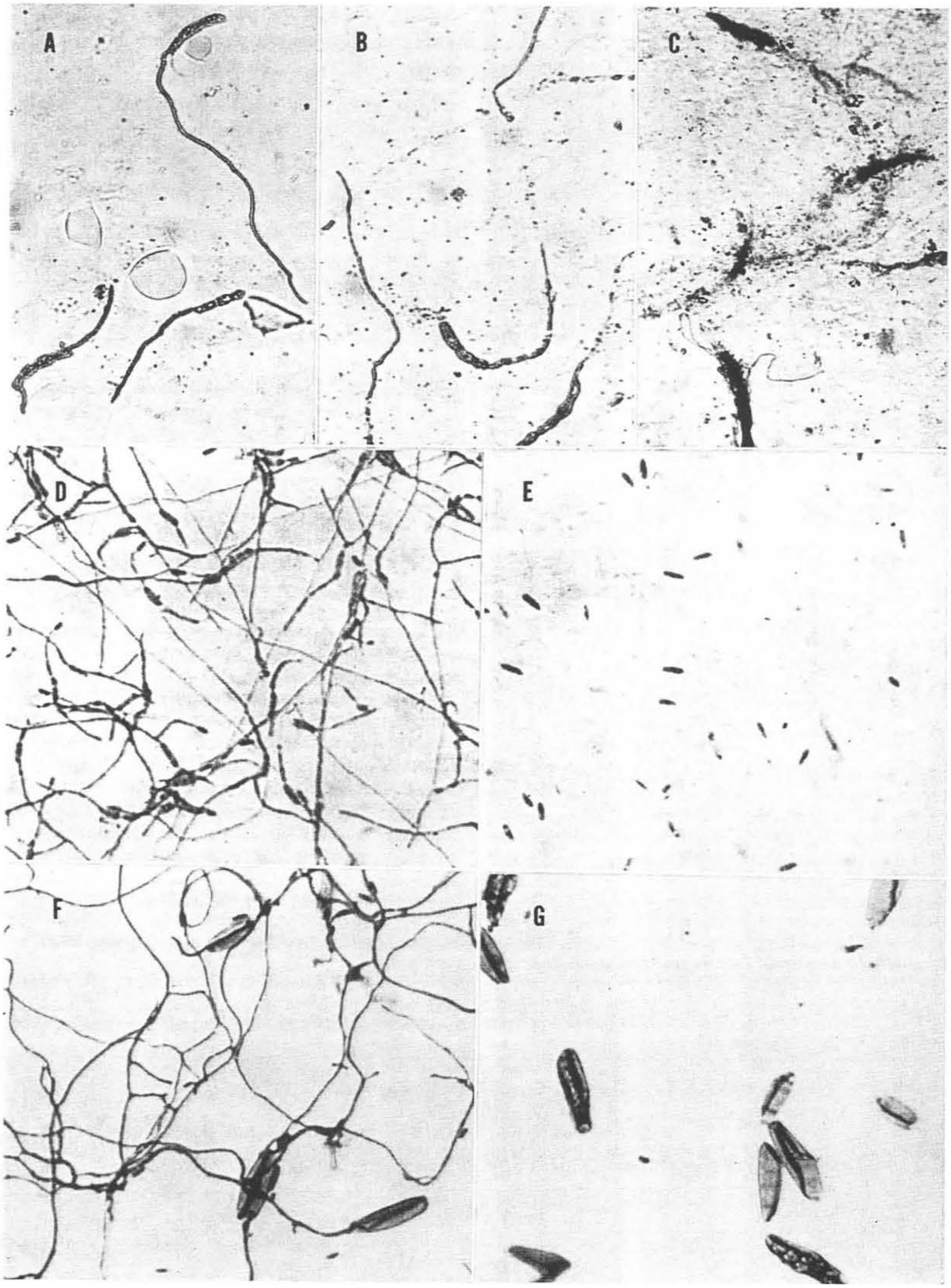
soil reinfested with specific microorganisms. Conover loam soil in 250-ml Erlenmeyer flasks was sterilized by autoclaving it for 40 min on 3 consecutive days, by exposure to propylene oxide gas for 16 hr in a closed container, or by gamma irradiation. With the last method, the soil in small glass jars was exposed to a dosage of 4 megarads from a Co^{60} source. This work was kindly supervised by H. G. Olson, Phoenix Memorial Laboratory, Ann Arbor, Mich. Sterilized soil was used in this condition or was infested with streptomycetes or bacteria and then incubated for 6-8 days at 24 C.

RESULTS.—*Lysis of living fungal hyphae on soil.*—Washed growing hyphae of the 10 test fungi, which included eight different species, were completely or partially lysed in 8 days on the surface of natural soil (Table 1). The sequence shown in Fig. 1—A,B,C, demonstrates the progressive lysis of hyphae of *F. solani* f. *phaseoli*. At 2 days the hyphae were lightly stained and the protoplasm frequently separated into segments. By 4 days many of the hyphae were lysed, especially the younger portions (Fig. 1-B). At 8 days lysis was complete, and bacteria and actinomycetes had developed in the region previously occupied by the fungus (Fig. 1-C). This sequence was also characteristic for lysis of most of the other fungi. Hyphae of *Glomerella cingulata* (Ston.) Spauld. & Schrenk were completely destroyed in 4 days and those of all of the other fungi except *Rhizoctonia* spp. were destroyed in 8 days. Hyphae of *Verticillium albo-atrum* Reinke & Berth., *Rhizoctonia solani* Kühn, and *R. praticola* Saksena & Vaartaja were more resistant to mycolysis than those of the other fungi, as previously noted (11). The dark pigmented conidiophores and the older mycelium of *Helminthosporium victoriae* Meehan & Murphy resisted lysis, whereas young hyphae lysed rapidly. *Rhizoctonia* spp. were the only fungi tested which grew on the soil. Thin, deeply stained, and sparsely branched hyphae spread over the soil in a linear pattern, but after 8 days much of this mycelium was lysed. Hyphal cells of *Mucor ramannianus* Möller, *Penicillium frequentans* Westling, and *V. albo-atrum* occasionally formed chlamydospores that persisted in the soil after lysis of the vegetative hyphae. Occasionally a cell from a nongerminated macroconidium of *Fusarium solani* f. *pisi* (F.R. Jones) Snyd. & Hans. or *F. solani* f. *phaseoli* rounded and became a chlamydospore, but hyphal cells rarely produced chlamydospores.

Differences in resistance to lysis were not obviously associated with pathogenic or nonpathogenic habit, nor was resistance to lysis associated with the type of propagule that produced the germ tube. Hyphae from chlamydospores of *F. solani* f. *phaseoli* lysed as rapidly as did hyphae from conidia. Lysis of both 1- and 6-day-old hyphae of several fungal species occurred at similar rates, indicating that within these limits hyphae of different ages do not differ appreciably in susceptibility to lysis.

All of several other soils tested lysed fungal hyphae, in confirmation of earlier work (10).

Fig. 1. A-C) (×305) Progressive stages in the lysis of *Fusarium solani* f. *phaseoli* on natural soil. A) 0 days: Germinated conidia were placed on the soil surface and recovered immediately. B) 4 days: Hyphae are partially disintegrated. C) 8 days: Hyphae have completely disappeared, leaving empty macroconidia, with abundant bacteria and actinomycetes in the regions previously occupied by the hyphae. **D-G)** (×205) Complete autolysis of fungal hyphae. D) *Glomerella cingulata* hyphae on a membrane filter before being placed on dialysis tubing. E) *G. cingulata* hyphae on a membrane filter that was placed on dialysis tubing inflated with circulating mineral salt solution, and the antibiotic filipin applied to the hyphae. After 2 days the hyphae were almost completely autolyzed, leaving conidia and some hyphal fragments. F, G) *Helminthosporium victoriae* hyphae that had received the same treatments as D and E.


A
B
C
D
E
F
G

Lysis of dead hyphae on soil.—Hyphae of the 10 test fungi were killed by exposure to 80 C for 4 min in a water bath. These hyphae lysed on the surface of natural soil, but at a slower rate than did living hyphae (Table 1). For example, living hyphae of *G. cingulata* were completely lysed in 4 days, whereas heat-killed hyphae were lysed in 8 days. However, hyphae of *G. cingulata* and *F. solani* f. *phaseoli* killed either by propylene oxide gas or by gamma radiation lysed on soil at the same rate as living hyphae. This may be related to the fact that hyphae partially autolyzed during the long treatment required for gas or radiation killing, whereas no autolysis occurred with heat killing.

Restoring the mycolytic property to sterilized soil with streptomycetes and bacteria.—Soil sterilized by autoclaving, propylene oxide gas, or radiation supported abundant mycelial growth of the 10 test fungi. Nine of 10 streptomycete isolates from soil restored the lytic property to sterilized soil when *G. cingulata* was used as the test organism. Combinations of mycolytic streptomycetes were no more effective than many of the single isolates. Of eight bacterial isolates from soil, two restored the lytic property to sterilized soil; the same two bacteria also inhibited germination of fungal conidia on agar, but did not hydrolyze colloidal chitin in agar.

Autoclaved soil infested with one of the streptomycete isolates was evaluated for ability to lyse hyphae of the 10 test fungi. All 10 fungi grew for a limited time on the streptomycete-infested soil, but subsequently all lysed as they would have in natural soil. When the fungi were ranked in order of increasing resistance to soil mycolysis, the order was the same for both streptomycete-infested soil and natural soil, although the rate of lysis was always faster on natural soil.

Organic nutrients reverse soil mycolysis.—Glucose or peptone, equivalent in amount to 0.2% of the dry weight of the soil, was added with live germinated spores of the test fungus to the surface of natural soil or a streptomycete-infested sterilized soil. Chitin was mixed with the soil at the same rate 4 days before addition of the fungus.

Glucose or peptone decreased the rate of lysis on both natural soil and streptomycete-infested soil by stimulating temporary fungus growth, whereas chitin increased the rate of mycolysis. For example, hyphae of *F. solani* f. *phaseoli* were completely lysed in 12 days on glucose- or peptone-supplemented soil, in 8 days on nonsupplemented natural soil, and in 4 days on soil supplemented with chitin. Corresponding results for *G. cingulata* were 8, 4, and 2 days, respectively. The results were similar on streptomycete-infested soil, but the rate of lysis was always slightly slower than on natural soil. Both glucose and peptone stimulated the development of large populations of native bacteria, actinomycetes, and fungi in natural soil, and of the streptomycete in streptomycete-infested soil. Mycelia of the test fungi coexisted for some time with the increased microbial population, but eventually lysed.

Soil microorganisms utilize products of lysing fungal mycelium in soil.—Hyphae of *G. cingulata* were added to moist soil in an amount equivalent in dry weight of mycelium to 1% of the dry weight of the soil. At intervals, estimates of the numbers of soil microorganisms per g dry weight of soil were made by the soil dilution plate method. For bacterial counts, the final soil dilution was 1×10^{-8} in soil extract agar (3), and for actinomycete counts, 1×10^{-6} in chitin agar (8). Six replicate plates were used.

In fungus-supplemented natural soil, the bacteria population increased approximately 65-70-fold in 5 days and the actinomycete population increased 10-fold in 9 days (Table 2). Fungal mycelium, as observed in stained smears of the soils and on plastic peelings, began to lyse on the second day and had completely disappeared by the fourth day. Concurrently bacterial numbers began increasing on the second day and continued to increase until the fifth day. Counts on the seventh day had not increased further. Numbers of actinomycetes did not increase until the fourth day. However, this lag in actinomycete numbers probably reflects a period of active mycelial growth prior to sporulation, since abundant actinomycete mycelia appeared on the surface of the soil on the third day. A

TABLE 2. Increases in the numbers of bacteria and actinomycetes in soil supplemented with living hyphae of *Glomerella cingulata* at the rate of 1% of the soil dry weight

Days after supplementing soil	Lysis rating[a]	Bacteria[b] Natural soil	Bacteria[b] Supplemented soil	Actinomycetes[b] Natural soil	Actinomycetes[b] Supplemented soil
		10^8 *colonies/g*		10^6 *colonies/g*	
0	0	1a	1a	6v	6v
1	0		1a		5v
2	2	2a	13b	6v	6v
3	3		28c		7v
4	4	2a	94d	5v	13w
5	4		126c		29x
7	4	2a	138c	5v	46y
9	4			6v	60z

[a] Lysis was rated on a scale from 0-4: 0 = no lysis; 1 = 1-10% of hyphae lysed; 2 = 10-50%; 3 = 50-90%; 4 = 90-100%.

[b] Each value is an average of six replicates. Numbers followed by the same letter do not differ significantly at the 1% level of probability.

large number of chitinolytic bacteria also appeared on the chitin agar plates.

Lysis of fungal hyphae separated from soil by a membrane filter.—The mechanism whereby mycolytic microorganisms induce the lysis of fungi is not known. Hyphae could lyse either by self-digestion from their own intracellular enzymes (autolysis) or by extracellular enzymes from soil microorganisms (heterolysis). If lysis of living fungal hyphae in soil is autolytic, then separating soil from fungal hyphae by a membrane that prevents passage of enzymes should not prevent mycolysis.

Natural soil was placed in a petri dish, and the surface was smoothed and covered with a washed, cold-sterilized membrane filter. The filters selected were 50 mm in diam and were composed of cellulose esters that resisted biological degradation. One type of filter was 130 μ thick with a designated pore size of 5 or 10 mμ (Millipore Corp., Bedford, Mass.). The other type used was 220 μ thick with a designated pore size of 4 mμ (Gelman Co., Ann Arbor, Mich.). Filters were incubated on the soil surface for 1 day, after which washed living or dead hyphae of the test fungi were placed on their upper surfaces, i.e., the filter separated the hyphae from the soil. As controls, filters with hyphae were placed on the surface of autoclaved soil. After 4-8 days of incubation in moist conditions at 24 C, filters were removed from the soil surface and washed. The mycelium was stained with rose bengal, and the filter was dried and mounted in immersion oil where it became transparent. The stained hyphae were observed with a microscope using bright-field illumination, or unstained preparations were examined by phase contrast.

Living hyphae of *G. cingulata* were almost completely lysed in 8 days on filters of all three pore sizes; only conidia and a few hyphal remnants remained (Table 3). Small hyphal fragments could be seen with phase contrast. Hyphae of *H. victoriae* were almost completely lysed in 8 days on the 130-μ filters and 50-90% lysed on the 220-μ filters. Hyphae of *F. solani* f. *phaseoli* grew on the filter surfaces, sporulated, and then partially lysed; after 8 days on the 130-μ filters, 50-90% of the hyphae were destroyed, leaving hyphal fragments, conidia, and scattered chlamydospores joined to discontinuous hyphal remnants. The mycelia of all three fungi grew abundantly over the surfaces of filters on autoclaved soil.

Soil was also placed on the surfaces of membrane filters that rested on living 3-day-old cultures of *G. cingulata* growing in 0.5% peptone agar. Partial lysis occurred beneath the 4, 5, and 10 mμ pore-size filters, which contained soil supplemented with water or colloidal chitin equivalent in amount to 0.2% of the soil weight. Little or no lysis occurred beneath filters containing soil supplemented at the same rate with glucose or peptone. Although the rate of lysis was decreased when filters were used, its characteristics closely resembled those of lysis occurring on the soil alone.

If lytic enzymes present in soil passed through the membrane filters and caused lysis of the living hyphae, then dead hyphae on membrane filters in contact with soil should also lyse. However, dead hyphae of the three test fungi showed no lysis on the 130-μ filters placed on soil for periods of time up to 12 days (Table 3).

When solutions of *Streptomyces* chitinase (9) were passed through the three filters, assays of the filtrates (9) never showed chitinase activity. Moreover, chitin was not degraded when membranes were placed on chitin agar and either natural soil or streptomycete cultures were placed on the upper surface. Thus, pore sizes of the filters were small enough to prevent the passage of large molecules such as enzymes.

No contaminating microorganisms were found by microscopic observations in the area of the lysed living fungal hyphae, and washings from the filters were sterile. Even if some contaminants were present, they were probably ineffective in causing mycolysis since hyphae grew without lysing on parts of the filter surface above air pockets trapped between the soil and the filter.

It was concluded that lysis of living fungal hyphae on the surface of membrane filters in contact with soil was due to autolysis, and not to enzymes from microorganisms in the soil.

Complete autolysis of fungal hyphae induced by combined starvation and antibiotic treatment.—Since starvation or the presence of toxic substances, or both, is considered responsible for the autolysis of fungi in staled cultures (5), these conditions were imposed on

TABLE 3. Lysis of fungal hyphae on membrane filters that separated the hyphae from soil

Filter		Condition of hyphae	Lysis rating after 4, 8, and 12 days[a]								
			Glomerella cingulata			*Helminthosporium victoriae*			*Fusarium solani*		
Pore size	Thickness		4	8	12	4	8	12	4	8	12
*m*μ	μ										
10	130	Living	4	4	4	2	4	4	2	3	4
		Dead	0	0	0	0	0	0	0	0	0
5	130	Living	3	4	4	2	4	4	2	3	4
		Dead		0	0		0	0		0	0
4	220	Living	3	4		2	3		2	2	

[a] Lysis was rated 0-4: 0 = no lysis; 1 = 1-10% of hyphae lysed; 2 = 10-50%; 3 = 50-90%; 4 = 90-100%. Values are from one of two experiments with similar results.

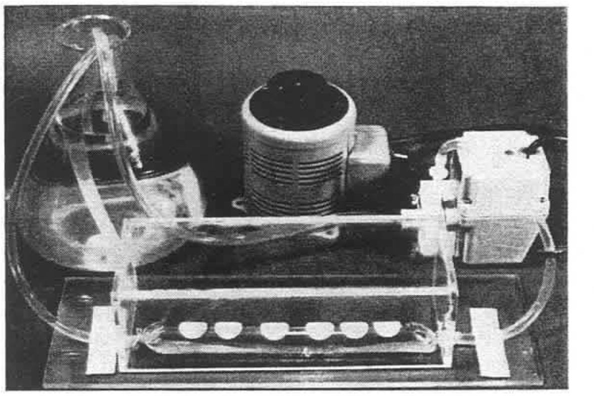


Fig. 2. Dialysis system. Fungal hyphae are on the outer surface of the membrane filters resting on the dialysis tubing. Pump at right circulates mineral salt solution through the dialysis tubing via the reservoir at left.

fungal hyphae in attempts to induce autolysis. Membrane filters (0.45 or 0.65 mμ pore size) containing young fungal hyphae were washed in dilute mineral salt solution and then placed on a sterilized inflated dialysis tubing (Fig. 2). After 3-4 hr of incubation, about 0.2 ml of a solution containing 100 μg/ml of an antifungal antibiotic in 1% ethanol was added to the surface of each filter. The application was repeated twice at about 3-hr intervals and twice again after 1 day. A centrifugal pump slowly circulated 10% Reynolds' mineral salt solution (18) through the dialysis tubing and into a 3-liter reservoir from which the solution was recirculated. The purpose of the dialysis system was to starve the hyphae by creating a diffusion gradient away from the fungus. Most organic or inorganic materials leaking from the hyphae would pass through the tubing and become diluted by the large volume of circulating solution. Temperature of the circulating solution was kept at 25 C. Sometimes streptomycin sulfate (40 μg/ml) was added to inhibit bacteria. Aseptic procedures were used where practical. On the second day, the filters were removed and the hyphae were stained and mounted in immersion oil for microscopic observation.

The antifungal antibiotics cycloheximide, endomycin, and filipin (Upjohn Co., Kalamazoo, Mich.) and nystatin (Squibb Inst., New Brunswick, N.J.) induced much autolysis of starved hyphae of *G. cingulata* and *H. victoriae* after 1 day. The remaining hyphae were lightly stained and the protoplasm frequently segmented. On the second day the hyphae of both fungi had almost completely lysed; only conidia, a few small hyphal fragments, and occasional hyphal tips of *H. victoriae* remained (Fig. 1-D-G). In unstained preparations viewed with phase contrast, only a few hyphal fragments could be seen. Partial lysis of hyphae of *F. solani* f. *phaseoli* occurred, the majority of the hyphae being lightly stained but apparently intact. Pimaricin (Royal Netherlands Fermentation Ind., Ltd., Delft) was less effective than the other antibiotics.

Contaminants are believed to have been absent or unimportant since very few bacteria were seen on the filters, and the use of streptomycin sulfate in the circulating solution did not interfere with lysis.

Starvation or antibiotic treatments applied separately resulted in only partial lysis of fungal hyphae. For example, cell walls of the three test fungi exposed to the dialysis system but without antibiotics remained intact, although the protoplasm of *G. cingulata* and *H. victoriae* became separated into segments. Application of several antibiotics, including those used above, to 3-day-old cultures of *G. cingulata* in 0.5% peptone agar likewise induced only partial autolysis. Similar results were reported previously (4). Washed hyphae of *F. solani* f. *phaseoli* also autolyzed only slightly during 22 hr of incubation in a solution of 100 μg/ml of endomycin or cycloheximide, and no lysis of *G. cingulata* was detected in the same antibiotics.

Occasionally, autolysis failed to occur with the antibiotic-starvation system. Varying conditions such as humidity within the Plexiglas chamber, temperature of the circulating solution, and type of membrane filter did not prevent the occasional failure.

Antibiotic production during lysis of fungal mycelium in natural soil.—If the starvation-antibiotic model is applicable to the soil, then antibiotics must be formed in the vicinity of fungal hyphae. Natural soil (40 g) was supplemented with washed living mycelium of *G. cingulata* in an amount equivalent in dry weight of the mycelium to 1% of the soil dry weight. After 2-4 days of incubation at 24 C, soil was extracted with 40 ml ethanol and centrifuged, and the supernatant fluid was evaporated to dryness under vacuum at 40 C. The residue was dissolved in 5 ml of ethanol and absorbed in filter paper discs, which were air dried and placed on the surface of 0.5% peptone agar containing a conidial suspension of either *G. cingulata* or *F. solani* f. *phaseoli*. The plates were incubated at 24 C for 1 or 2 days.

In three of five tests, extracts from fungus-supplemented soil formed large and well-defined inhibition zones, indicating the presence of an antibiotic. In the other two tests no inhibition zones were formed. No inhibition occurred with discs containing ethanol extracts of *G. cingulata* mycelium or of nonsupplemented natural soil.

DISCUSSION.—These results confirm previous results from this laboratory on the existence and microbial origin of mycolytic activity in soils (10, 11, 12). Rather than employing agar cultures of test fungi as in our previous work, the present work made use of a direct method which involved only soil and washed hyphal suspensions of test fungi.

Lysis of fungal mycelium in soil could be caused either by extracellular enzymes liberated by soil microorganisms (heterolysis) or by intracellular enzymes which result in self-digestion of the fungal mycelium (autolysis). A clear distinction between these alternatives seems fundamental to an understanding of the mechanism of soil mycolysis. If extracellular enzymes lyse fungal mycelium, one of these enzymes would have to be chitinase, as chitin is a major constituent of fungal cell walls. Some evidence suggests a causal

role for chitinase and other enzymes in mycolysis. Most of the mycolytic actinomycetes and bacteria examined produced chitinase (6, 9, 14, 15), and some lytic bacteria produce β-D-1,3-glucanase (14) which hydrolyzes another major constituent of the cell walls of filamentous fungi. Dead mycelium or cell wall preparations were lysed by crude or partially purified preparations containing chitinase or other hydrolytic enzymes from *Streptomyces* or bacterial cultures (6, 9, 14, 15, 19). Living mycelia of *Aspergillus oryzae* (6) and *G. cingulata* (15) were also partially lysed by crude enzyme preparations, but the temperature used in both of these cases was 37 C, which is probably lethal for these fungi. At 30 C, a more favorable temperature for fungal growth, an enzyme preparation from *Streptomyces* sp. caused extrusion of the protoplasm from hyphae of several fungi, presumably by dissolution of the cell wall in restricted areas (1). However, the relevance of this phenomenon to the more generalized and complete lysis occurring in soil is uncertain. The heterolytic point of view has been sponsored most actively by Alexander and his colleagues (2, 13, 14, 19).

Against the circumstantial evidence for heterolysis rests the fact that extracellular enzymes thus far have not been demonstrated to lyse living fungal hyphae at temperatures favorable for fungus growth. Chitinase, either alone or in combination with other enzymes known to degrade constituents of fungal cell walls, did not lyse living mycelium at 24 or 28 C (9, 15). Further, ability to produce chitinase does not seem obligatory in lytic microorganisms. In the present work two bacterial isolates that did not produce extracellular chitinase nonetheless restored the mycolytic property to sterilized soil. Moreover, the amount of fungal mycelium lysed by mycolytic actinomycetes in agar tests bore no relation to the amount of chitinase they produced on chitin agar (9). A final decision as to whether extracellular hydrolytic enzymes of soil microorganisms lyse cell walls of living fungi must await additional research.

Evidence in this report, however, suggests that an autolytic mechanism can account for soil mycolysis. When soil was separated from fungal mycelium by membrane filters having pores small enough to prevent passage of enzymes, living hyphae of several fungi were completely or partially lysed whereas dead hyphae were not lysed. Finally, complete autolysis of living hyphae of *G. cingulata* or *H. victoriae* was induced by exposure to antifungal antibiotics when the hyphae were in a starved condition. To our knowledge, this is the first report of mycolysis characteristic of that in soil occurring in the absence of microorganisms at temperatures favorable for fungal growth.

Although complete autolysis of hyphae of *G. cingulata* and *H. victoriae* took place with the starvation-antibiotic system, autolysis of *F. solani* f. *phaseoli* was incomplete. Lysis of this fungus was also incomplete on membrane filters in contact with soil. Since hyphae of *F. solani* f. *phaseoli* lysed completely in contact with soil, complete destruction of some fungi in soil may require secondary degradation by extracellular enzymes from other microorganisms. This may explain why ability to lyse and ability to produce chitinase are frequently combined in the same lytic microorganism. Natural selection would favor those organisms that can both induce autolysis and compete for cell-wall remnants by formation of such extracellular enzymes as chitinase.

In contrast with our results that lysis of fungal mycelium can occur through a membrane capable of obstructing the passage of enzymes, Mitchell (13) reported that lysis did not occur when dialysis tubing (regenerated cellulose) was placed between fungal mycelium and soil. This was given as evidence that hydrolytic enzymes produced by lytic microorganisms are necessary for lysis of fungi in soil. We repeated this experiment using a different test fungus, *G. cingulata*, and obtained the same results. However, we propose an alternative interpretation. In our experiment the dialysis tubing was partially degraded both by the test fungus and by microorganisms in the soil. Possibly hydrolytic products were available as nutrients for the fungus, thereby precluding lysis. Certainly, the cellulose did not long remain an effective barrier to the passage of enzymes.

There is some evidence to support the starvation-antibiotic model for soil mycolysis. (i) The course and characteristics of lysis of living hyphae with this system were similar to those of lysis in the soil. (ii) Lytic ability in agar was restricted to streptomycetes that also produced antibiotics (9, 12); further, amount of living mycelium lysed and degree of inhibition of fungal growth by the same streptomycetes were significantly correlated (9). (iii) Fungal hyphae are lysed when organic nutrients available to the fungus are deficient, and nutrients, particularly carbon, in the bulk of soil free from decomposing organic matter are known to be scarce. (iv) An antifungal substance was extracted from soil supplemented with living fungal hyphae.

Still another view of the mechanism of lysis has been put forward by Payen (17). He believes that hyphae are first killed by a preformed toxin in soil, after which the protoplasm leaks from the hyphae. This is followed by destruction of the empty hyphae by extracellular enzymes. Although this model has not been evaluated experimentally, our results indicate that lysis of hyphal walls occurs concurrently with disappearance of protoplasm. Moreover, there is no clear evidence that preformed soil toxins cause death of fungi in soil.

Finally, we suggest the following tentative working hypothesis for soil mycolysis. In the presence of organic nutrients available to the fungus, soil fungistasis is revoked. Fungal propagules germinate and colonize the new substrate coexisting temporarily with large populations of other microorganisms. With the exhaustion of nutrients through competition, the mycelium becomes starved. Antibiotics are formed by microorganisms utilizing soluble nutrients leaking from fungal hyphae, or possibly from nutrients in the original substrate. The antibiotics together with starvation conditions induce autolysis of the mycelium.

The hyphae of some fungi are completely destroyed by autolysis but hyphae of other fungi are only partially autolyzed. Final dissolution of the remaining cell walls would then depend on extracellular enzymes, which in soil come from other microorganisms.

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